Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2007.03573.x

Factors associated with CD4 lymphocyte counts in HIV-negative Senegalese individuals

C. Mair,* S. E. Hawes,* H. D. Agne,† P. S. Sow,† I. N'doye,‡ L. E. Manhart,* P. L. Fu,§ G. S. Gottlieb¶ and N. B. Kiviat**

*Department of Epidemiology, School of Public Health, Departments of ¶Medicine and **Pathology, School of Medicine, University of Washington, Seattle, WA, §Lumetra, San Francisco, CA, USA, †Department of Infectious Diseases, University of Dakar, and ‡Institut d'Hygiene Sociale, Dakar, Senegal

Accepted for publication 11 November 2007
Correspondence: S. E. Hawes, University of Washington HPV Research Group, 1914 North 34th Street, Suite 300, Seattle, WA 98103, USA. E-mail: hawes@u.washington.edu

Summary

CD4+ lymphocytes are a primary target of the human immunodeficiency virus (HIV), and CD4 counts are one of the factors used to measure disease progression in HIV-positive individuals. CD4 counts vary in uninfected individuals and across populations due to a variety of demographic, environmental, immunological and genetic factors that probably persist throughout the course of HIV infection. This study sought to determine reference levels and identify factors that influence lymphocyte counts in 681 HIV-uninfected adults in Senegal, where residents are exposed to a variety of infectious diseases and other conditions that may affect CD4 counts. Lymphocyte counts were assessed in commercial sex workers, symptomatic men and women presenting to the University of Dakar infectious disease clinic for out-patient care and women seeking family planning services. CD4 and CD3 lymphocyte counts differed between the four study groups ($P < 0{\cdot}01$). Men had the lowest mean CD4 count (711·6 cells/μl), while commercial sex workers had the highest levels (966·0 cells/μl). After adjustment for age and other behavioural and clinical factors, the difference in CD4 counts between the three groups of women did not remain. However, both gender and smoking were associated independently with CD4 counts, as men maintained lower mean CD4 counts ($\beta = -156{\cdot}4$ cells/μl, $P < 0{\cdot}01$) and smokers had higher mean CD4 counts ($\beta = 124{\cdot}0$ cells/μl, $P < 0{\cdot}01$) than non-smokers in multivariable analyses. This study is the first to explore factors that may influence CD4 levels in Senegal and to estimate baseline CD4 levels among HIV-negatives, information that may guide clinicians in interpreting CD4 counts.

Keywords: CD4 counts, commercial sex work, HIV, infectious diseases, Senegal

Introduction

CD4, CD8 and other lymphocytes co-ordinate the immune system's response to pathogens [1]. In human immunodeficiency virus (HIV)-uninfected individuals a CD4 count, measured in cells per cubic millilitres of blood, provides a picture of immune system health, with higher CD4 counts typically signifying healthier immune systems. Children in both developed and developing countries are born with high CD4 counts, which decline slowly through adolescence and then plateau [2].

Underlying demographic and genetic factors, current exposure to infectious diseases and behavioural factors have been associated with variations in CD4 cell counts in HIV-negative populations [3]. Healthy African and Asian populations typically have lower CD4 lymphocyte counts than their western European and Caucasian counterparts [3–5], but data from specific countries are limited. Paradoxically, cigarette smoking has been associated with higher CD4 counts in several studies [6–11]. Underlying infectious diseases, such as pneumonia and tuberculosis (TB), have been associated with decreased CD4 levels [12,13]. Commercial sex workers (CSW), who are exposed typically to a wide variety of sexually transmitted infections, have somewhat lower lymphocyte counts than females who are not involved in the sex trade [14,15]. In western populations, black race, low body mass index (BMI) and injection drug use have also been associated with lower CD4 lymphocyte counts [13,16], and women tend to have CD4 levels 1–200 cells/μl higher than men with comparable demographic and behavioural patterns [9,17].

The CD4 lymphocyte count of HIV-infected individuals is an important predictor of HIV disease progression and acquired immune deficiency syndrome (AIDS)-associated mortality [18–20]. In HIV infection, CD4 cell loss is associated with an increased risk of developing opportunistic infections and AIDS-associated malignancies. CD4 count, along with HIV viral load and clinical symptoms, is used to determine when to start highly active anti-retroviral therapy (HAART). Current World Health Organization (WHO) recommendations for initiating anti-retroviral therapy in HIV-infected individuals include WHO clinical stage IV (clinical AIDS) regardless of CD4 count, clinical stage III with CD4 count < 350 cells/μl or CD4 count below 200 cells/μl regardless of clinical stage [21]. Although, to date, data suggest that the natural history of HIV disease progression (i.e. time from infection to clinical AIDS) is similar in developed and developing countries [22], whether lower baseline, pre-HIV infection CD4 counts in some populations in developing countries with high rates of malnutrition and non-HIV infectious disease burdens account for reports of more rapid progression to AIDS remains to be explored further [23].

To date there are no published data on CD4 counts in healthy Senegalese or on factors associated with lower CD4 levels among HIV-negative Senegalese. To estimate baseline CD4 counts in HIV-negative Senegalese populations and to identify factors associated with lower CD4 counts among various subpopulations, we conducted a secondary cross-sectional data analysis of clinic attendees in four different HIV-negative subgroups: (i) female commercial sex workers; (ii) men presenting to an infectious disease (ID) clinic for out-patient care with symptoms of an infectious disease; (iii) women presenting to an ID clinic for out-patient care with symptoms of an infectious disease; and (iv) women visiting an ID clinic for family planning services (e.g. birth control, standard gynaecological visit). We also investigated the demographic and clinical correlates of CD4 counts within each group of HIV-negative men and women. Finally, we determined whether between-group differences in CD4 counts were explained by the demographic and clinical factors associated with CD4 counts in the study.

Materials and methods

Individuals were recruited from three different study sites in Senegal between October 1994 and January 1998 as part of studies investigating the epidemiology of HIV, human papillomavirus (HPV), cervical neoplasia and oral manifestations of HIV, as described previously [20,24–26]. The majority of male and female study participants were recruited from an out-patient infectious disease clinic at the Fann Hospital of the University of Dakar, while registered female commercial sex workers were enrolled from two sexually transmitted disease (STD) clinics in Dakar and M'Bour during required visits. All participants provided informed consent and those presenting to the Fann Hospital were offered a dental (oral) examination in addition to HIV screening. Women were also offered Papanicolaou (Pap) screening for detection of cervical neoplasia. When appropriate, medication was given to treat underlying infections, although information regarding the specific treatments was not obtained. At the initial screening visit, 6784 men and women were screened serologically for HIV infection and 5638 men and women were determined to be HIV-negative. Of these, 850 HIV-negative subjects were enrolled into longitudinal studies. CD4 and other lymphocyte data were obtained for 681 (80%) of the 850 HIV-negative enrolled individuals at the enrolment visit, including 120 men and 376 women from the out-patient infectious disease clinic and 185 CSW from the two STD clinics. Median time from screening to enrolment was 56 days. At enrolment, demographic information, medical and sexual histories and blood samples were collected and all participants underwent a general physical examination, where signs and symptoms and presumptive diagnoses were recorded. Blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes and was analysed using the fluorescence activated cell sorter (FACS)Count analyser (Becton-Dickinson Biosciences, San Jose, CA, USA) to determine the number of CD4, CD8 and CD3 cells/μl. Informed consent was obtained according to procedures approved by the Human Subjects Committee of the University of Washington and the Senegalese National AIDS Committee.

The study population comprised four distinct groups: HIV-negative men symptomatic for an infectious disease; HIV-negative women symptomatic for an infectious disease; HIV-negative asymptomatic women visiting the clinic for family planning-related activities; and asymptomatic HIV-negative CSW attending an STD clinic for routine screening. We used analysis of variance (ANOVA) to calculate univariate statistics for lymphocyte counts (CD4, CD8, CD3, CD8 : CD4 ratio and CD4%), stratified by study group, comparing all four groups at once to eliminate the potential problem of multiple comparisons. Duncan and Waller tests were utilized to determine whether lymphocyte counts were different at the $P < 0{\cdot}05$ level between each pair of groups.

To assess factors associated with CD4 count levels within groups, two types of univariate analyses were performed: one for all study subjects and separate parallel analyses within each study group. Continuous variables were divided into groups, and cut-off points for the group categories were based on biologically relevant values (e.g. Centers for Disease Control classifications for healthy body size for BMI categories). Alternatively, groups of roughly equal size were formed if there was no a priori reason for a given cut-off point.

The extent of variation in lymphocyte counts and/or risk factors by subpopulation group was assessed in four multivariate models using linear regression, adjusting for a variety of factors. The first model compared the effect of membership in each of the four study groups on CD4 counts, with the reference group consisting of symptomatic women who

Table 1. Comparison of demographic, clinical and behavioural characteristics of Senegalese men and women visiting an infectious disease clinic, women seeking family planning services and female commercial sex workers [*n** (%)].

	Men (ID clinic) $n = 120$	Women (ID clinic) $n = 210$	Women (FP) $n = 166$	CSW $n = 185$	All $n = 681$	P-value†
Age						
Mean (±s.d.)	31·6 (±11·2)	31·5 (±8·1)	32·5 (±7·6)	29·9 (±7·0)	31·3 (±8·4)	0·03
Marital status						
Married	43 (36·8)	144 (70·6)	138 (83·6)	6 (3·2)	331 (49·4)	< 0·001
Level of schooling						
Primary	11 (40·7)	34 (48·6)	34 (54·0)	27 (50·9)	106 (49·8)	0·17
Alcohol use						
Yes	6 (5·0)	6 (2·9)	1 (0·6)	57 (30·8)	70 (10·3)	< 0·001
Current tobacco use						
Yes	35 (29·2)	13 (6·2)	11 (6·6)	93 (50·3)	152 (22·3)	< 0·001
Place of birth						
Not Senegal	11 (9·1)	10 (4·9)	10 (6·0)	44 (24·0)	75 (11·1)	< 0·001
Number of children						
Mean (±s.d.)	n.a.	3·5 (±3·2)	4·6 (±3·2)	2·5 (±2·5)	3·5 (±3·1)	< 0·001
Contraceptive method						
None	n.a.	141 (68·1)	56 (33·9)	29 (15·8)	226 (40·6)	< 0·001
Condoms	n.a.	11 (5·3)	8 (4·8)	111 (60·3)	130 (23·4)	
Other BC	n.a.	55 (26·6)	101 (61·2)	44 (23·9)	200 (36·0)	
Vaginal discharge						
Yes	n.a.	185 (96·9)	147 (93·0)	96 (54·2)	428 (81·4)	0·004
Cervicitis						
Yes	n.a.	41 (21·5)	44 (27·8)	4 (2·2)	89 (16·7)	< 0·001
Sex with non-Senegalese						
Yes	n.a.	16 (8·6)	19 (12·3)	88 (51·8)	123 (24·2)	< 0·001
Current tuberculosis						
Yes	10 (9·5)	14 (7·8)	4 (2·8)	1 (0·6)	29 (5·0)	0·002
Current pneumonia						
Yes	13 (12·1)	11 (5·7)	7 (4·4)	2 (1·1)	33 (5·2)	< 0·001
Age of first intercourse						
Mean (±s.d.)	18·7 (±4·9)	18·2 (±4·0)	18·0 (±4·1)	16·3 (±2·9)	17·7 (±4·0)	< 0·001
Body mass index						
Mean (±s.d.)	20·0 (±5·9)	22·9 (±5·8)	23·0 (±4·6)	22·8 (±5·2)	22·3 (±5·5)	< 0·001
Temperature (°C)						
38+	16 (15·2)	17 (9·1)	18 (12·2)	8 (4·6)	59 (9·6)	0·01

*n is smaller than listed in table heading for most variables, due to missing values. †P-values compare the distribution of each variable between the four subpopulations.

visited the infectious disease clinic, given their direct comparability to each of the other three groups. Next, multivariate models were developed to identify factors that were associated independently with an individual's CD4 count, adjusting for other relevant characteristics identified a priori from the literature (tobacco use, age, age at first intercourse and body temperature) and from among characteristics that were associated significantly with CD4 counts in univariate analyses. Separate models with all study subjects, males only and females only were evaluated.

Results

Men attending the Fann University Hospital out-patient infectious disease clinic presented for a variety of reasons, including known or suspected HIV infection (15%), diarrhoea (11%) and urethral discharge (5%) [26], while women attended for family planning (26%), general gynaecological problems (17%), perceived infertility (8%) and genital infections (8%). The most commonly reported clinical symptoms were headache, nausea and fever. The mean age of study participants was 31·3 and differed somewhat by study group ($P = 0·03$) (Table 1). Most (63%) men were not married, while the vast majority of non-CSW women were currently married and most CSW (59%) were divorced ($P < 0·01$). Alcohol use was rare (<5%) in all but the CSW study group (31%; $P < 0·01$). Men (29%) and CSW (50%) were more likely to be current smokers than women presenting to the clinic for family planning (7%) or infectious disease (6%; $P < 0·01$) services. As expected, subjects visiting the clinic for

Table 2. Comparison of mean lymphocyte counts among human immunodeficiency virus-negative Senegalese men and women.

	Men (ID clinic) mean (s.d.) $n = 120$	Women (ID clinic) mean (s.d.) $n = 210$	Women (FP) mean (s.d.) $n = 166$	CSW mean (s.d.) $n = 185$	All mean (s.d.) $n = 681$	P-value*
CD4	711·6 (332·8)	851·9 (325·0)	903·1 (320·4)	966·0 (366·4)	870·7 (347·0)	< 0·001
CD8	540·5 (284·5)	501·1 (265·1)	484·8 (223·0)	539·1 (267·7)	514·7 (260·5)	0·14
CD3	1336·0 (534·8)	1435·7 (526·8)	1477·1 (495·3)	1592·5 (573·2)	1471·0 (540·1)	< 0·001
CD4% (CD4/CD3)	0·53 (0·14)	0·60 (0·10)	0·62 (0·09)	0·61 (0·10)	0·59 (0·11)	< 0·001
CD4 : CD8 ratio	1·49 (0·68)	1·91 (0·74)	2·08 (0·09)	1·99 (0·10)	1·90 (0·77)	< 0·001

*P-value compares the mean of all four groups, using analysis of variance.

infectious diseases reported higher rates of TB, pneumonia and weight loss than asymptomatic women.

The overall mean CD4 count of all study participants was 870·7 cells/μl, although CD4 counts differed significantly between the four study groups (Table 2, $P < 0·01$). Men had the lowest mean CD4 count (711·6 cells/μl), while that in all women studied was 904·7 cells/μl ($P < 0·01$). Mean CD4 counts differed somewhat between groups of women ($P = 0·09$), and were lowest in women attending ID clinics (851·9 cells/μl) compared to women seeking family planning services (903·1 cells/μl) and CSW (966·0 cells/μl). Overall, 86 HIV-negative individuals (12·6%) had CD4 counts below 500 cells/μl, including 44 (6·5%) below 350 cells/μl and 13 (1·9%) below 200 cells/μl. CD8 counts were similar between the four groups ($P = 0·14$), but CD3 counts, which are comprised mainly of CD4 and CD8 cells, varied by group ($P < 0·01$). CD3 counts were highest in commercial sex workers (mean 1592·5 cells/μl) and lowest in men (mean 1336·0 cells/μl), mirroring the relationship observed with CD4 counts. CD4% and CD4 : CD8 ratios were similar in the three groups of women, with mean CD4% ranging from 60 to 62% and mean ratios from 1·91 to 2·08, but were lower in men attending the ID clinic (53% and 1·49, respectively; $P < 0·01$ for both comparisons).

In univariate analyses, male gender was associated with significantly lower CD4 counts compared to female gender (mean difference, 193·1 cells/μl, $P < 0·01$, Table 3). Young study subjects (< 20) had somewhat lower CD4 counts than subjects in their 20s (mean difference 65·1; $P < 0·01$). Other demographic characteristics, such as ethnic group (data not shown), level of schooling and place of birth, were not associated significantly with CD4 level. Smoking was associated with a CD4 count increase of 115·6 cells/μl ($P < 0·01$). Increased height (> 175 cm) and having a low BMI (< 18) were both related to a significantly lower CD4 count ($P < 0·05$). Individuals who reported current TB had mean CD4 counts 231·3 cells/μl lower than those without TB, and those with a current self-reported diagnosis of pneumonia had CD4 counts that were 175·8 cells/μl lower than those without ($P < 0·01$ for both comparisons). Symptoms of underlying illness, such as high body temperature ($\beta = -149·6$, $P = 0·02$), relatively high systolic blood pressure ($\beta = -119·2$, $P = 0·04$) and swollen lymph nodes ($\beta = -66·4$, $P = 0·01$), were all associated with significantly lower average CD4 count. In women, abnormal cervical findings, including discharge ($\beta = -119·0$, $P < 0·01$) and cervicitis ($\beta = -75·8$, $P = 0·05$), were also associated with lower CD4 count.

In multivariate analyses adjusting simultaneously for age, age of first intercourse and body temperature, male gender remained associated with lower CD4 count ($\beta = -156·4$ cells/μl, $P < 0·01$, Table 4, model 1), while tobacco use was associated with a 124·0 cells/μl ($P < 0·01$) higher CD4 count. Substantial differences between symptomatic women presenting to the ID clinic (reference group), women presenting for family planning ($\beta = 47·3$ cells/μl, $P = 0·21$) and female CSW ($\beta = 15·2$ cells/μl, $P = 0·71$) were not observed. Male gender was associated with lower CD4 counts both in smokers ($\beta = -186·8$) and non-smokers ($\beta = -166·2$, data not shown). In parallel multivariable analyses, smoking had a similar and significant effect on CD4 counts in females (model 2, $\beta = 105·2$) and males (model 3, $\beta = 186·5$), and an interaction term between smoking and gender was non-significant ($P = 0·31$).

Discussion

This study is the first to present normal reference ranges for HIV-uninfected Senegalese men and women. The mean CD4 count in this group of Senegalese men and women was 871 cells/μl, and differed between men (mean = 712 cells/μl) and women (mean = 905 cells/μl) ($P < 0·01$). Higher CD4 counts were associated independently with female gender and tobacco use while lower CD4 counts were associated independently with later age at sexual debut and elevated body temperature. Among women, no association was observed between CD4 counts and number of children or contraceptive use. Overall mean CD4 counts were higher in CSW than in women seeking care for symptomatic infectious disease or family planning in univariate analyses, although the difference in CD4 counts between these three groups of women was attenuated after controlling for behavioural and clinical characteristics. CD4 counts among the men (all of whom were symptomatic) remained significantly lower

Table 3. Univariate analysis of factors associated with CD4 counts among human immunodeficiency virus-negative Senegalese men and women.

Variable	β*	*P*-value
Gender		
Male	−193·1	< 0·01
Marital status		
Single	Ref.	Ref.
Monogamously married	53·2	0·13
Polygamously married	7·9	0·85
Other	69·0	0·07
Age group (years)		
< 20	−65·1	< 0·01
20–29	Ref.	Ref.
30–39	−38·3	0·20
40+	−55·7	0·15
Level of schooling		
Primary	Ref.	Ref.
Secondary	65·7	0·53
College	135·1	0·13
Place of birth		
In Senegal	Ref.	Ref.
Out of Senegal	−9·4	0·83
Alcohol use		
Yes	67·3	0·12
Current tobacco use		
Yes	115·6	< 0·01
Age of first intercourse (years)		
≤15	−35·1	0·32
16–18	Ref.	Ref.
19+	−96·8	< 0·01
Weight (kg)		
< 51	18·6	0·63
51–60	Ref.	Ref.
61–70	67·0	0·07
71+	78·2	0·05
Height (cm)		
<165	57·6	0·09
165–174	Ref.	Ref.
175+	−95·2	0·02
Body mass index (kg/m^2)		
< 18·5	−104·1	< 0·01
18·5–24·9	Ref.	Ref.
25–29·9	30·5	0·51
30+	2·2	0·97
Temperature (°C)		
< 37	Ref.	Ref.
37–37·9	−76·3	0·14
38+	−149·6	0·02
Systolic blood pressure		
< 100	Ref.	Ref.
100–130	−25·3	0·57
130+	−119·2	0·04
Diastolic blood pressure		
< 70	Ref.	Ref.
70–80	−29·7	0·55
80+	−69·9	0·14
Current TB		
Yes	−231·3	< 0·01
Cough		
Yes	−24·3	0·64
Diarrhoea > 1 month		
Yes	−170·1	0·12
Weight loss		
Yes	−46·9	0·11
Current pneumonia		
Yes	−175·8	< 0·01
Swollen lymph nodes		
Yes	−66·4	0·01
Oral leucoplacia		
Yes	−264·0	0·27
Systolic blood pressure		
< 100	Ref.	Ref.
100–130	−25·3	0·57
Characteristics in females (n = 561)		
Number of children		
0	44·6	0·34
1–3	Ref.	Ref.
4–7	20·5	0·55
8+	−19·1	0·68
Contraceptive method		
None	Ref.	Ref.
Condoms	49·8	0·18
OCs	−33·1	0·44
Other	−39·9	0·31
Abnormal discharge		
Yes	−119·0	< 0·01
Cervicitis		
Yes	−75·8	0·05
Pregnant		
Yes	−190·1	0·43

*β for difference in mean CD4 count compared to reference group, from univariate linear regression analysis. Reference group not listed or designated by (Ref).

(156·4 cells/μl) than those of symptomatic women in multivariate analyses.

CD4 counts in non-smoking women in this Senegalese population were somewhat lower than those reported recently in healthy adults in more developed nations such as Italy and the United States, but are higher than those reported in countries such as India, Botswana and Singapore (Fig. 1) [27–43]. Similarly, CD4 counts in Senegalese men in the current study, who all presented to an out-patient ID clinic with symptoms of infectious diseases, were generally lower than those observed in other populations, although Senegalese men who smoked had high CD4 counts compared to most populations. This variation across studies and geographical region may be explained partially by differences in study populations, such as age, race/ethnicity, the proportion of individuals who smoke and prevalence of underlying diseases, all of which have been shown to be associated with differences in CD4 counts [5–13,43]. Despite geographical differences in baseline CD4 levels, studies of

Table 4. Multivariate analysis of factors associated with CD4 counts among human immunodeficiency virus-negative Senegalese men and women.

	Model 1: all subjects $n = 561$		Model 2: women only $n = 465$		Model 3: men only $n = 93$	
Variable	β	*P*-value	β	*P*-value	β	*P*-value
Group 1 (men)	−156·4	< 0·01	–	–	–	–
Group 3 (FP women)	47·3	0·21	47·8	0·22	–	–
Group 4 (CSW)	15·2	0·71	35·4	0·48	–	–
Tobacco use	124·0	< 0·01	105·2	0·02	186·5	0·01
Age*	−1·6	0·34	−2·9	0·31	−3·0	0·33
Age of first intercourse†	−7·7	0·04	−5·5	0·24	−6·7	0·43
Temperature‡	−86·9	0·02	−72·7	0·07	−126·8	0·12
Birth control method: condom	–	–	−5·9	0·90	–	–
Birth control method: oral contraception	–	–	−46·0	0·32	–	–
Number of children	–	–	6·1	0·41	–	–

Reference values: 0 children, birth control method: none/methods besides condoms and oral contraception. *Per increasing year above age 14. †Per increasing year above age 10. ‡Per increasing 1°C temperature above 36°C.

HIV-infected individuals have concluded that the lower CD4 cell counts often noted in HIV-negative individuals in developing nations are not associated with differing natural histories of HIV infection [44,45].

In comparison to other African countries, Senegal has relatively low rates of sexually transmitted infections, in part because the government of Senegal requires CSW to register and receive annual STD testing. Untreated STDs have been associated with a decline in CD4 cell counts in HIV-positive individuals, which return to baseline upon treatment [46]. In the present study, in univariate analyses, we observed that commercial sex workers had significantly higher CD4 counts than did women presenting to an outpatient clinic for symptoms of infectious diseases ($\beta = 114{\cdot}1$; $P < 0{\cdot}01$) or for family planning ($\beta = 62{\cdot}9$; $P = 0{\cdot}09$). However, this difference was attenuated after adjusting for other factors, such as smoking, in a multivariate model ($\beta = 15{\cdot}2$; $P = 0{\cdot}71$). In a small study in Southern India, Babu *et al.* [15] did not observe any difference in CD4 counts between HIV-negative CSW ($n = 37$) and normal healthy female controls ($n = 35$), but did observe higher CD8 cells counts and lower CD4 : CD8 ratios in CSW. In contrast, we observed similar CD8 counts in CSW and non-CSW women.

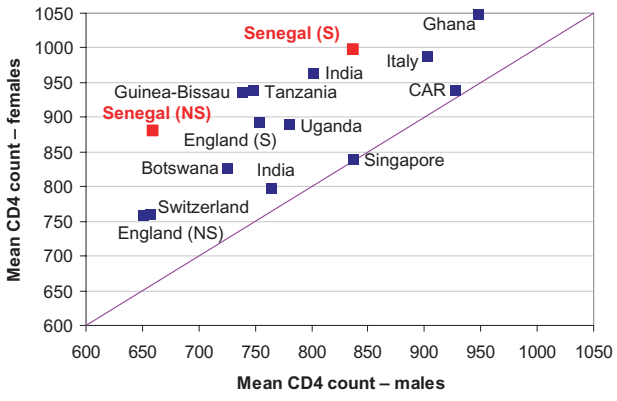

Fig. 1. Mean CD4 counts, by gender (and smoking), in Senegal and other study populations.

We found a substantial difference in CD4 levels between men and women in this study (156·4 cells/μl), even after adjustment for other important factors, including smoking. While this is consistent with most previously published studies assessing gender differences, which report a 80–200 cell/μl larger average CD4 count in women compared to men (Fig. 1) [9,16,28–32,35,38,41,47,48], in some populations no significant differences have been observed in CD4 levels by gender [36,49–52]. Differences between studies may be due in part to a lack of adjustment for confounding due to other important risk factors, such as age and cigarette smoking. The importance of gender differences in baseline CD4 cell counts, especially in terms of potential differences in progression to AIDS and the timing of initiation of antiretroviral therapy, is still debated [17,53–56], but currently these potential gender differences in baseline CD4 counts have not translated into changes in clinical practice.

The association between smoking and increased CD4 count in healthy populations is well established. Our study found differences similar to those reported previously [6,28], although this difference has not been universal [48]. In HIV-infected individuals, smoking may be associated with poorer virological and immunological responses [57] and increased comorbidity and mortality [57,58], although these effects appear to be independent of the effect of smoking on CD4 counts. In univariate analyses, increased height and lower body mass index were associated significantly with a lower CD4 count in the current study population, similar to observations in Ethiopia and a US population [7,43]. BMI is, to some degree, a surrogate for the strong effect of male gender, as Senegalese men in the study were taller, had lower average BMI and had lower CD4 counts than Senegalese women. In univariate analyses stratified by gender, being underweight remained somewhat associated with lower CD4 counts,

although this association was not significant in either males or females. However, due to large numbers of missing data, BMI was not assessed in the final multivariable analyses.

In multivariate analysis, smoking and gender accounted for the majority of the between-group differences we observed in unadjusted analyses, with male gender exercising the greatest influence on mean CD4 count. Smoking was associated similarly with increased CD4 counts in both men and women. The observation that, among women, only smoking was associated independently with higher CD4 levels while differences between the subpopulation groups of women were no longer associated with CD4 count suggests that these factors, rather than any inherent differences between the groups of women, are responsible for the differing crude CD4 levels between commercial sex workers and other clinic attendees. Among men, the only factor that was associated independently with CD4 counts was smoking. Thus, Senegalese clinicians may need to consider both gender and smoking status when interpreting a patient's CD4 count. These risk factors, irrespective of geographical location, appear to influence individuals' CD4 levels substantially.

Utilizing diverse study populations with a wide range of exposure histories and demographic patterns, this study is the first to explore factors that may influence CD4 levels in Senegal, and to estimate baseline CD4 count levels among HIV-negative Senegalese. Use of an extensive questionnaire allowed us to examine both variables that have previously been linked to CD4 level, such as smoking, as well as to explore other potential correlates of CD4 count such as sexual history and birth control method. Nevertheless, our study population is not representative of the entire country of Senegal: due to the clinic-based study population, study subjects were more likely to have an infectious disease than are those in the general population, and due to the aims of the primary studies, subjects had higher rates of abnormal oral screening examinations and genital HPV infections than are seen typically in the general population. Abnormal screening examinations might signal an underlying infectious disease which, in turn, may influence lymphocyte counts. Thus, the observed mean CD4 counts in these groups may be underestimates of general population levels. Many of the characteristics we evaluated were derived from self-report, which may have biased our assessment of their relationship to CD4 count, as a layperson probably has a different perception of health problems than a physician. Furthermore, we had no information on the time of day that the serum specimen was drawn. Previous studies have demonstrated significant diurnal variation in lymphocyte levels, and such variation may have biased the CD4 measurements [13].

The reference ranges presented offer clinicians a baseline for CD4 counts in Senegalese men and women prior to infection with HIV. Because CD4 levels in HIV-negative Senegalese are relatively high, WHO guidelines for initiating anti-retroviral therapy (ART) are probably appropriate for this country. However, a variation of 100 cells/μl in HIV-positive patients can influence assignment to a given stage of HIV disease progression and/or clinical AIDS diagnosis, altering the recommendations for initiation of ART. The mean difference between genders in this Senegalese population was greater than 150 cells/μl, with women having significantly higher CD4 counts than men. A recent meta-analysis concluded that women have lower plasma HIV RNA levels than men for a given CD4 count [56]. However, as there is no strong evidence to suggest that gender affects HIV disease progression [55], the biological relevance of differences in baseline CD4 counts between men and women remains unknown. Similarly, the mechanism behind the difference in CD4 count levels in smokers and non-smokers is poorly understood. A meta-analysis of 10 studies in the 1990s concluded that there is little evidence that tobacco smoking is related to progression to AIDS [59], although more recent studies suggests that smoking may be related to poorer response to anti-retroviral therapy and higher risk of death post-HAART [57,58]. None the less, these reference ranges and knowledge of patient gender and smoking status may be helpful to Senegalese clinicians attempting to interpret a CD4 count.

Acknowledgements

We thank Mame Birame Diouf, Deana Rich, Elise Reay-Ellers and Macoumba Touré for their invaluable co-ordination of study procedures in Senegal, Aissatou Diop, Mame Dieumbe Mbengue-Ly, Marie Pierre Sy and Dr Pierre Ndiaye for patient care, Fatou Faye-Diop for data entry, Jane Kuypers for coordination of the Seattle laboratory and Alison Starling for forms development and data management. This study was supported by grants from the National Cancer Institute (CA 62801 and CA 97275).

References

1 Koenig S, Fausi A. Immunology of human immunodeficiency virus infection. In: Holmes KK, Mardh P, Sparling PA, Lemon SM, eds. Sexually transmitted diseases, 3rd edn. New York: McGraw-Hill 1999:231–49.

2 Tsegaye A, Wolday D, Otto S *et al.* Immunophenotyping of blood lymphocytes at birth, during childhood, and during adulthood in HIV-1-uninfected Ethiopians. Clin Immunol 2003; **109**:338–46.

3 Clerici M, Butto S, Lukwiya M *et al.* Immune activation in Africa is environmentally-driven and is associated with upregulation of CCR5. Italian–Ugandan AIDS Project. AIDS 2000; **14**:2083–92.

4 Howard RR, Fasano CS, Frey L, Miller CH. Reference intervals of CD3, CD4, CD8, CD4/CD8, and absolute CD4 values in Asian and non-Asian populations. Cytometry 1996; **26**:231–2.

5 Messele T, Abdulkadir M, Fontanet AL *et al.* Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clin Exp Immunol 1999; **115**:443–50.

6 Tollerud DJ, Clark JW, Brown LM *et al.* The effects of cigarette smoking on T cell subsets. A population-based survey of healthy Caucasians. Am Rev Respir Dis 1989; **139**:1446–51.

7 Abuye C, Tsegaye A, West CE *et al.* Determinants of CD4 counts among HIV-negative Ethiopians: role of body mass index, gender, cigarette smoking, khat (*Catha edulis*) chewing, and possibly altitude? J Clin Immunol 2005; **25**:127–33.

8 Schaberg T, Theilacker C, Nitschke OT, Lode H. Lymphocyte subsets in peripheral blood and smoking habits. Lung 1997; **175**:387–94.

9 Maini MK, Gilson RJ, Chavda N *et al.* Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. Genitourin Med 1996; **72**:27–31.

10 Tanigawa T, Araki S, Nakata A *et al.* Increase in memory (CD4+CD29+ and Cd4+CD45RO+) T naive (CD4+CD45RA+) T-cell subpopulations in smokers. Arch Environ Health 1998; **53**:378–83.

11 Sunyer J, Munoz A, Peng Y *et al.* Longitudinal relation between smoking and white blood cells. Am J Epidemiol 1996; **144**:734–41.

12 Fantin B, Joly V, Elbim C *et al.* Lymphocyte subset counts during the course of community-acquired pneumonia: evolution according to age, human immunodeficiency virus status, and etiologic microorganisms. Clin Infect Dis 1996; **22**:1096–8.

13 Aldrich J, Gross R, Adler M, King K, MacGregor RR, Gluckman SJ. The effect of acute severe illness on CD4+ lymphocyte counts in nonimmunocompromised patients. Arch Intern Med 2000; **160**:715–16.

14 Messele T, Rinke de Wit TF, Brouwer M *et al.* No difference in *in vitro* susceptibility to HIV type 1 between high-risk HIV-negative Ethiopian commercial sex workers and low-risk control subjects. AIDS Res Hum Retroviruses 2001; **17**:433–41.

15 Babu PG, Pramilabai A, Sripriya G, Damodharan S, John TJ. Immunologic profiles of HIV-infected and uninfected commercial sex workers in the Vellore region of Southern India. J Acquir Immune Defic Syndr Hum Retrovirol 1997; **16**:357–61.

16 Tollerud DJ, Clark JW, Brown LM *et al.* The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. J Clin Immunol 1989; **9**:214–22.

17 Prins M, Robertson JR, Brettle RP *et al.* Do gender differences in CD4 cell counts matter? AIDS 1999; **13**:2361–4.

18 Mellors JW, Munoz A, Giorgi JV *et al.* Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997; **126**:946–54.

19 Benito JM, Lopez M, Lozano S *et al.* Differential upregulation of CD38 on different T-cell subsets may influence the ability to reconstitute CD4+ T cells under successful highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2005; **38**:373–81.

20 Gottlieb GS, Sow PS, Hawes SE *et al.* Equal plasma viral loads predict a similar rate of CD4+ T cell decline in human immunodeficiency virus (HIV) type 1- and HIV-2-infected individuals from Senegal, West Africa. J Infect Dis 2002; **185**:905–14.

21 UNAIDS/WHO. Epidemiological Fact Sheets on HIV/AIDS and Sexually Transmitted Infections, 2006 Update. World Health Organization, Geneva, 2006. Report generated August 2007. Available at: http://www.who.int/globalatlas/predefinedReports/EFS2006/EFS_PDFs/EFS2006_SN.pdf (accessed August 2007).

22 Glynn JR, Sonnenberg P, Nelson G, Bester A, Shearer S, Murray J. Survival from HIV-1 seroconversion in Southern Africa: a retrospective cohort study in nearly 2000 gold-miners over 10 years of follow-up. AIDS 2007; **21**:625–32.

23 Williams BG, Korenromp EL, Gouws E, Schmid GP, Auvert B, Dye C. HIV infection, antiretroviral therapy, and CD4+ cell count distributions in African populations. J Infect Dis 2006; **194**:1450–8.

24 Hawes SE, Critchlow CW, Faye Niang MA *et al.* Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections. J Infect Dis 2003; **188**:555–63.

25 Hawes SE, Critchlow CW, Sow PS *et al.* Incident high-grade squamous intraepithelial lesions in Senegalese women with and without human immunodeficiency virus type 1 (HIV-1) and HIV-2. J Natl Cancer Inst 2006; **98**:100–9.

26 Sow PS, Hawes SE, Critchlow CW *et al.* Characteristics and presenting complaints of outpatients with undiagnosed HIV infection: potential utility in selecting subjects for HIV testing. J Acquir Immune Defic Syndr 2004; **37**:1520–8.

27 Tsegaye A, Messele T, Tilahun T *et al.* Immunohematological reference ranges for adult Ethiopians. Clin Diagn Lab Immunol 1999; **6**:410–14.

28 Santagostino A, Garbaccio G, Pistorio A *et al.* An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica 1999; **84**:499–504.

29 Bisset LR, Lung TL, Kaelin M, Ludwig E, Dubs RW. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur J Haematol 2004; **72**:203–12.

30 Urassa WK, Mbena EM, Swai AB, Gaines H, Mhalu FS, Biberfeld G. Lymphocyte subset enumeration in HIV seronegative and HIV-1 seropositive adults in Dar es Salaam, Tanzania: determination of reference values in males and females and comparison of two flow cytometric methods. J Immunol Methods 2003; **277**:65–74.

31 Norrgren H, Da Silva ZJ, Andersson S *et al.* Clinical features, immunological changes and mortality in a cohort of HIV-2-infected individuals in Bissau, Guinea-Bissau. Scand J Infect Dis 1998; **30**:323–9.

32 Bussmann H, Wester CW, Masupu KV *et al.* Low CD4+ T-lymphocyte values in human immunodeficiency virus-negative adults in Botswana. Clin Diagn Lab Immunol 2004; **11**:930–5.

33 Attili VS, Sundar S, Singh VP, Rai M. Validity of existing CD4+ classification in north Indians, in predicting immune status. J Infect 2005; **51**:41–6.

34 Ampofo W, Torpey K, Mukadi YD *et al.* Normal CD4+ T lymphocyte levels in HIV seronegative individuals in the Manya/Yilo Krobo communities in the Eastern region of Ghana. Viral Immunol 2006; **19**:260–6.

35 Lugada ES, Mermin J, Kaharuza F *et al.* Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol 2004; **11**:29–34.

36 Aina O, Dadik J, Charurat M *et al.* Reference values of CD4 T lymphocytes in human immunodeficiency virus-negative adult Nigerians. Clin Diagn Lab Immunol 2005; **12**:525–30.

37 Levin A, Brubaker G, Shao JS *et al.* Determination of T-lymphocyte subsets on site in rural Tanzania: results in HIV-1 infected and non-infected individuals. Int J STD AIDS 1996; **7**:288–91.

38 Zekeng L, Sadjo A, Meli J *et al.* T lymphocyte subset values among healthy Cameroonians. J Acquir Immune Defic Syndr Hum Retrovirol 1997; **14**:82–3.

39 Adurogbangba MI, Aderinokun GA, Odaibo GN, Olaleye OD, Lawoyin TO. Oro-facial lesions and CD4 counts associated with

HIV/AIDS in an adult population in Oyo State. Nigeria Oral Dis 2004; **10**:319–26.

40 Jiang W, Kang L, Lu HZ *et al.* Normal values for CD4 and CD8 lymphocyte subsets in healthy Chinese adults from Shanghai. Clin Diagn Lab Immunol 2004; **11**:811–13.

41 Amatya R, Vajpayee M, Kaushik S, Kanswal S, Pandey RM, Seth P. Lymphocyte immunophenotype reference ranges in healthy Indian adults: implications for management of HIV/AIDS in India. Clin Immunol 2004; **112**:290–5.

42 Vithayasai V, Sirisanthana T, Sakonwasun C, Suvanpiyasiri C. Flow cytometric analysis of T-lymphocytes subsets in adult Thais. Asian Pac J Allergy Immunol 1997; **15**:141–6.

43 Nowicki MJ, Karim R, Mack WJ *et al.* Correlates of CD4+ and CD8+ lymphocyte counts in high-risk immunodeficiency virus (HIV)-seronegative women enrolled in the women's interagency HIV study (WIHS). Hum Immunol 2007; **68**:342–9.

44 Morgan D, Mahe C, Mayanja B, Okongo JM, Lubega R, Whitworth JA. HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? AIDS 2002; **16**:597–603.

45 Mekonnen Y, Geskus RB, Hendriks JC *et al.* Low CD4 T cell counts before HIV-1 seroconversion do not affect disease progression in Ethiopian factory workers. J Infect Dis 2005; **192**:739–48.

46 Anzala AO, Simonsen JN, Kimani J *et al.* Acute sexually transmitted infections increase human immunodeficiency virus type 1 plasma viremia, increase plasma type 2 cytokines, and decrease CD4 cell counts. J Infect Dis 2000; **182**:459–66.

47 Reichert T, DeBruyere M, Deneys V *et al.* Lymphocyte subset reference ranges in adult Caucasians. Clin Immunol Immunopathol 1991; **60**:190–208.

48 Uppal SS, Verma S, Dhot PS. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. Cytometry B Clin Cytom 2003; **52**:32–6.

49 Menard D, Mandeng MJ, Tothy MB, Kelembho EK, Gresenguet G, Talarmin A. Immunohematological reference ranges for adults from the Central African Republic. Clin Diagn Lab Immunol 2003; **10**:443–5.

50 Nag VL, Agarwal P, Venkatesh V, Rastogi P, Tandon R, Agrawal SK. A pilot study on observations on CD4 & CD8 counts in healthy HIV seronegative individuals. Indian J Med Res 2002; **116**:45–9.

51 Chng WJ, Tan GB, Kuperan P. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. Clin Diagn Lab Immunol 2004; **11**:168–73.

52 Kelly P, Zulu I, Amadi B *et al.* Morbidity and nutritional impairment in relation to CD4 count in a Zambian population with high HIV prevalence. Acta Trop 2002; **83**:151–8.

53 Delmas MC, Jadand C, De Vincenzi I *et al.* Gender difference in CD4+ cell counts persist after HIV-1 infection. SEROCO Study Group. AIDS 1997; **11**:1071–3.

54 Moore AL, Kirk O, Johnson AM *et al.* Virologic, immunologic, and clinical response to highly active antiretroviral therapy: the gender issue revisited. J Acquir Immune Defic Syndr 2003; **32**:452–61.

55 Nicastri E, Angeletti C, Palmisano L *et al.* Gender differences in clinical progression of HIV-1-infected individuals during long-term highly active antiretroviral therapy. AIDS 2005; **19**:577–83.

56 Napravnik S, Poole C, Thomas JC, Eron JJ Jr. Gender difference in HIV RNA levels: a meta-analysis of published studies. J Acquir Immune Defic Syndr 2002; **31**:11–19.

57 Feldman JG, Minkoff H, Schneider MF *et al.* Association of cigarette smoking with HIV prognosis among women in the HAART era: a report from the women's interagency HIV study. Am J Public Health 2006; **96**:1060–5.

58 Crothers K, Griffith TA, McGinnis KA *et al.* The impact of cigarette smoking on mortality, quality of life, and comorbid illness among HIV-positive veterans. J Gen Intern Med 2005; **20**:1142–5.

59 Furber AS, Maheswaran R, Newell JN, Carroll C. Is smoking tobacco an independent risk factor for HIV infection and progression to AIDS? A systemic review. Sex Transm Infect 2007; **83**:41–6.

